ELEVATED CYSTATIN-C CONCENTRATION AND PROGRESSION TO PREDIABETES: THE WESTERN NEW YORK STUDY

Richard P. Donahue, PhD[1], Saverio Stranges, MD[1,3], Karol Rejman MS[1] , Lisa B. Rafalson MS[1], Jacek Dmochowski, PhD[1,2], Maurizio Trevisan MD, MS[1]

[1]*Department of Social and Preventive Medicine, SUNY at Buffalo, Buffalo, NY*

[2]*Department Mathematics and Statistics, UNC Charlotte, Charlotte, NC*

[3]*Clinical Science Research Institute, Warwick Medical School, Coventry, UK*

This study was supported by a grant from NIH NIDDK RO1 DK60587.

Correspondence and Reprints to:

Richard P. Donahue, PhD, MPH

Department of Social & Preventive Medicine

School of Public Health and Health Professions

State University of New York at Buffalo

3435 Main Street, Farber Hall, Room 268 F

Buffalo, NY 14214 (USA)

Telephone: (716) 829.2975 ext. 614

Fax: (716) 829.2979

Email: rpd1@buffalo.edu

Short title: Cystatin-C and prediabetes

Word count of manuscript: 2731

Word count of abstract: 240

OBJECTIVE – We conducted a nested case-control investigation to examine if elevated baseline concentrations of cystatin-C predicted progression from normoglycaemia to prediabetes over 6 years of follow-up from the Western New York Health Study.

RESEARCH DESIGN AND METHODS – 1,455 participants from the Western New York Health Study, free of type 2 diabetes and known cardiovascular disease at baseline (1996-2001), were reexamined in 2002-2004. An incident case of prediabetes was defined as one with fasting glucose below 100 mg/dl at the baseline examination and ≥ 100 mg/dl and ≤ 125 mg/dl at the follow-up examination. All cases (n=91) were matched 1:3 to control participants based upon sex, race/ethnicity and year of study enrollment. All controls had fasting glucose levels < 100 mg/dl at both baseline and follow-up examinations. Cystatin-C concentrations and the urinary albumin to creatinine ratio were measured from frozen (-196 Cº) baseline blood and urine samples. Serum creatinine concentrations were available from the baseline examination.

RESULTS –Multivariate conditional logistic regression analyses adjusted for age, baseline glucose level, HOMA-IR, body mass index, hypertension, eGFR, cigarette smoking, and alcohol use revealed a significantly increased risk of progression to prediabetes among those with elevated baseline concentrations of cystatin-C (Odds Ratio, 95% CI: 3.04, 1.34, 6.89) (upper quintile vs. the remainder). Results of secondary analyses that considered hs-CRP, IL-6, E-selectin, or sICAM did not alter these results.

CONCLUSIONS - These results suggest that early renal impairment indexed with cystatin-C imparted a three-fold excess risk of progression to prediabetes in this study population.

Recent evidence from randomized clinical trials (1,2) among people with prediabetes have provided convincing evidence that early intervention can significantly delay or prevent the progression to type 2 diabetes. The identification of those with prediabetes is assuming greater importance (3) especially in light of the fact that approximately 35 million adults aged 40-74 years old in the United States have prediabetes defined as impaired fasting glucose (4). Microalbuminuria occurs frequently in nondiabetic subjects and places them at increased risk for cardiovascular disease (5-7). The mechanisms behind this observation are poorly understood, however. Albuminuria may reflect underlying vascular damage (8), hypertension (9, 10) endothelial dysfunction (11, 12) and/or low-grade inflammation (13).

A large percentage of type 2 individuals pass through a period of prediabetes (14) and may experience early renal dysfunction e.g., a glomerular filtration rate (GFR) above 60 ml/minute per 1.73m^2. Currently used estimating equations are poor at identifying early renal impairment and better indices are of great interest (15, 16). Recently, several studies have suggested that cystatin-C levels may be a more sensitive marker of early renal impairment than either albuminuria or serum creatinine concentration (17-20). Therefore, a better understanding of a putative role for cystatin-C in the etiology of prediabetes could shed light on the renal/heart disease connection (21). Given the reported superiority of cystatin C over conventional measures of renal function, we hypothesized that cystatin-C would predict progression to prediabetes independent of serum creatinine or estimated GFR. We also investigated the role of intervening

mechanisms including hypertension, insulin resistance, endothelial dysfunction and inflammation.

RESEARCH DESIGN AND METHODS

Participants

The study design and methodology of this community-based investigation have been previously published (22, 23). Briefly, participants in the this report were originally enrolled as healthy control subjects in the Western New York Study, an epidemiologic case-control investigation of alcohol intake patterns and risk of cardiovascular disease in Erie and Niagara Counties, New York conducted in 1996-2001. The initial cohort of control participants was randomly selected from drivers license lists for those under age 65, and from the Health Care Finance Administration rolls for those aged 65-79. In 2001-2004 we conducted the first follow-up of the apparently healthy MI control group. Eligible participants for the follow-up study were men and women aged 39-79 years selected from the baseline examination without known clinical cardiovascular disease (self-reported MI, angina or revascularization surgery) or type 2 diabetes mellitus (measured fasting plasma glucose > 125 mg/dl or self report and taking medication) and who were capable of completing the current study protocol (n=2652). Exclusion criteria also included self-report of a medical condition that would prohibit participation (e.g., all cancers except skin cancer, type 1 diabetes, physical or mental impairment), permanent change in residence out-of-state, deceased, or inability to contact and determine eligibility. This left 2139 persons eligible for

this examination of whom 1455 completed the full clinic protocol (68.0% response rate). Compared to those that refused, the participants were less likely to smoke and have more formal education. There were no significant differences in fasting glucose concentrations or BMI or sex ratio (data not presented). The mean follow-up time was 5.9 years (SD = 0.8 yrs). The protocol was approved by the University at Buffalo Health Science Institutional Review Board and all participants provided written informed consent prior to participation.

Study Protocol

At both the baseline and six-year follow-up examinations all participants received a clinical examination that included resting blood pressure, measures of height, weight, and waist girth according to standardized protocols (24). Hypertension was defined as a systolic pressure greater than or equal to 140 mmHg or a diastolic pressure greater than or equal to 90 mmHg or use of antihypertensive medications regardless of blood pressure level. Study subjects also provided a fasting (at least 10 hrs overnight) blood sample and asked to refrain for 24 hours from smoking or vigorous physical activity. Several standardized questionnaires were administered including cigarette use (never, ever) and lifetime pack-years of smoking, physical activity (Stanford 7-Day Recall), alcohol use, general health and well-being, personal and family health history, medication use and socioeconomic status. Participants were instructed to bring all medications to the clinic visits permitting identification of oral medications as well as insulin use. A positive family history of type 2 diabetes was defined as a positive report in a first

degree relative. An incident case of prediabetes was defined as one with fasting glucose below 100 mg/dl at the baseline examination and ≥ 100 mg/dl and ≤ 125 mg/dl at the follow-up examination. All cases (n=91) were matched 1:3 to control participants based upon sex, race/ethnicity and year of study enrollment. All controls had fasting glucose levels < 100 mg/dl at both baseline and follow-up examinations.

Laboratory Methods

Fasting glucose concentrations were determined by the glucose oxidase method (Beckman instruments, Fullerton, CA). The interasssay coefficient of determination was below 5%. After identification of those who progressed (cases) or not (controls) to prediabetes, the baseline aliquots of serum or plasma were retrieved and sent by overnight courier for analysis. Cystatin-C was measured using the BN II nephelometer (Dade Behring Inc., Deerfield, IL) utilizing a particle enhanced immunonepholometric assay (N Latex Cystatin C). This assay range was 0.195 to 7.330 mg/dl. The intra-assay CV% was 2.0-2.8% and the inter-assay range was 2.3-3.1%. Interleukin-6 was measured by ultra-sensitive ELISA (R&D Systems, Minneapolis, MN. Using this method, we have determined a routine CV in the lab of 6.3%. Soluble E-Selectin (E-Selectin) was measured using a high sensitivity quantitative sandwich enzyme (Parameter Human sE-Selectin Immunoassay; R&D Systems, Minneapolis, MN). Intra-assay and inter-assay CVs range from 4.7 – 5.0% and 5.7 – 8.8%, respectively. Human Soluble Intercellular Adhesion Molecule-1(sICAM) was measured by an

ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). The laboratory CV was 5.0%. High sensitivity C-reactive protein was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonepholometric assay. Intra-assay CVs range from 2.3 – 4.4% and inter-assay CVs range from 2.1 – 5.7%. Fasting insulin was assayed from a kit provided by Linco Research, Inc that has minimal cross reactivity with human proinsulin. The assay has a lower detection limit of 2 μU/ml with interassay CV% of 3.6-8.4% and intrassay CV% from 2.2-4.4%. The homeostasis model of insulin resistance (HOMA-IR) was calculated as fasting glucose x fasting insulin /22.4 (25). A frozen spot urine was also retrieved and utilized for the assessment of the albumin to creatinine (ACR) ratio. Collections were done in the morning of the baseline visit and stored on liquid nitrogen. Albumin was assayed with a nephelometric immunoassay using a monospecific antiserum to human albumin. Creatinine concentration was determined using the modified Jaffe method. The urinary albumin to creatinine ratio (ACR mg albumin / g creatinine) was calculated and utilized as a surrogate of albumin excretion rate (26). Intraclass correlation coefficients among duplicate samples exceeded 0.95. Over ninety-four percent of the values were below 30 mg/g. Glomerular filtration rate was estimated (eGFR) using the four variable formula from the Modification of Dietary Restriction in Diabetes Study (27, 28). This formula is: eGFR = 186.3 x (Serum creatinine concentration $^{-1.154}$) x (age$^{-0.203}$) x 1.212 (if black) x 0.742 (if female).

Statistical Procedures

For this report, data were compared between cases and controls with chi-square tests or unpaired t-tests as appropriate. Multivariate conditional logistic regression models (conditioned on the matching variables) were utilized to estimate the effect of elevated cystatin-C on prediabetic status after adjustment for age (model 1) and also after adjustment for several explanatory variables (model 2). We a priori compared the top quintile of cystatin-C to the remaining 80 percent determined by the distribution of cystatin-C among the controls. The top quintile range was 1.02-1.70 mg/dl. For ACR, we chose a cutpoint of 20, and for serum creatinine and eGFR, we split the distribution at the median value of the controls (0.8 mg/l and 83.3 ml per minute per 1.73 m^2). There were too few people to meaningfully examine the effect of higher clinical cutpoints. Likelihood ratio tests were conducted to test for statistical interactions by comparing the log likelihood between the two nested models, one with only the main effects, and the other with both the main effects and the interaction terms in the model. No significant effect-modification was noted. All statistical tests were two-sided and a p-value of < 0.05 was considered statistically significant. Analyses were carried out using the SPSS for Windows version 14.0 (Chicago, IL).

RESULTS

Table 1 presents selected characteristics at baseline for cases and controls. There were 91 cases of prediabetes that were matched with 273 control participants. Those who progressed to become cases of prediabetes were significantly older, more obese and heavier lifetime smokers than the controls ($P < 0.05$ for each). Cases were also significantly more insulin resistant at baseline and displayed higher mean concentrations of cystatin-C. No significant differences were observed for serum creatinine or eGFR. Fasting plasma glucose was higher among the cases than controls (94.3 vs 90.5 mg/dl; $P < 0.001$). Cases had significantly less formal education and a higher prevalence of a family history of diabetes, and were more likely to be hypertensive at the baseline examination. Compared to the controls, cases were more likely to have been smokers consumers of alcohol ($P < 0.05$).

Table 2 presents the results of conditional logistic regression also adjusted for age. As shown, a positive family history of type 2 diabetes, hypertension, cigarette smoking, and HOMA-IR were each related to prediabetic status. A BMI ≥ 27 kg/m^2 was associated with a 62 percent excess risk, although the 95% CI included unity. Neither serum creatinine nor ACR were found to predict prediabetic status. Consideration of continuous forms for these variables also provided no evidence of association with prediabetic status.

The results of the multiple conditional logistic regression analyses for cystatin-C and odds of developing prediabetes are displayed in Table 3. Models were conditioned on the matching variables of sex, race, and year of study enrollment. Model one was further adjusted for age and model 2 was fully-adjusted for age and several of the explanatory variables shown in table 2 including eGFR. Comparing the highest quintile of cystatin-C to the lowest 80% revealed an age-adjusted odds ratio of 5.08 (95%CI: 2.69, 9.58). After consideration of the putative risk factors for prediabetes in model 2, the odds ratio was attenuated to 3.04 (95% CI: 1.34, 6.89) but remained highly statistically significant ($P \leq 0.01$). No change in the effect size for cystatin-C was observed when serum creatinine (or ACR) was substituted for eGFR.

In order to investigate possible intervening mechanisms, we examined the association between cystatin-C, serum creatinine, urinary ACR, and several biomarkers of inflammation (hs-CRP and IL-6) and endothelial dysfunction (E-selectin and sICAM) among the control group (table 4). As shown, cystatin-C was significantly, though modestly associated with hs-CRP, E-selectin, sICAM, and IL-6 ($P < 0.001$ for each). Cystatin-C was not significantly correlated with ACR ($r_s = -0.007$) but was significantly correlated with serum creatinine ($r_s = 0.446$; $P < 0.001$) and eGFR ($r_s = -0.384$; $P < 0.01$). Secondary analyses were performed where biomarkers of inflammation or endothelial dysfunction were each considered one by one. Neither adjustment for E-selectin, nor sICAM had virtually any effect on the results.

Consideration of hs-CRP, IL-6 or eGFR likewise failed to materially alter the original findings.

CONCLUSION

In this investigation, we found an approximate three-fold increased risk of progression to prediabetes among those in the highest quintile of cystatin-C in a population free of diabetes and known cardiovascular disease. This association was independent of obesity, baseline glucose, eGFR (or serum creatinine), ACR, and other explanatory variables. These results extend previous observations for cardiovascular disease (17-20, 29) to include the early diabetic stage.

Several studies have examined proteinuria or microalbuminuria in relation to future health events in populations with type 1 diabetes, type 2 diabetes, and apparently healthy subjects. Proteinuria is a risk factor for end-stage renal disease among those with type 1 diabetes, and microalbuminuria has been associated with increased CVD mortality among both nondiabetic and type 2 diabetic persons (30-33). Cystatin C is also raised in the diabetic state but has been related to increased risk of mortality independent of diabetic status (18).

The results of this study support the hypothesis that cystatin – C may be elevated in advance of the onset of clinical diabetes and further suggest a preclinical stage of renal impairment that presages or develops in parallel with

the prediabetic condition (34). Our results also document the predictive power of cystatin C in the context of other risk factors for hyperglycemia and indicate that cystatin-C was a more powerful predictor than the traditional measures of serum creatinine or the ACR in a population with modest renal impairment. It is well known, however, that the estimated GFR is less precise among those with a GFR above 60 ml per minute per 1.73 m^2 of body surface area and thus has a limited prognostic value in a generally healthy population such as ours, thus we can not rule out that imprecision in the eGFR may still confound these results. Serum creatinine values also have limited power to accurately reflect underlying renal function among those with only modest impairment of kidney disease. These findings suggest that cystatin-C may be a more useful marker of renal impairment in populations with mild renal impairment.

The prevalence of hypertension at baseline was higher among the future case group than the matched controls. We do not know whether blood pressure rose before or concomitantly with cystatin C levels. Cigarette smoking and intensity of smoking were also higher among cases than controls. Smoking has been identified in some studies to predict microalbuminuria among type 1 individuals (30). Hyperinsulinemia/insulin resistance has also been associated with albuminuria in several reports (31- 33). Our results confirmed these associations but suggest further that the effect of cystatin C on risk of progression was largely independent of hypertension, smoking and HOMA-IR.

Preclinical kidney disease has been suggested to be related to underlying processes unrelated to the kidney per se (34). Our data indicated that cystatin-C was significantly correlated with several markers of inflammation including hs-CRP and IL-6. However in secondary analyses that considered these biomarkers, the results remained unaltered. The same held true for markers of endothelial function (E-selectin and sICAM (data not shown). These results are consistent with findings from the Cardiovascular Health Study (35) but suggest further that the effect of cystatin-C on risk of progression to prediabetes cannot be solely explained by inflammation or endothelial dysfunction. We did not find an association between urinary ACR and cystatin-C, likely due to the narrow distribution of ACR in our study population and/or the use of a single casual urine sample.

Several limitations are deserving of comment. We did not use an oral glucose tolerance test thus some of the study participants classified as prediabetic cases at the follow-up examination, may have undetected type 2 diabetes (36). However, fasting glucose measures are more highly correlated over time than the 2-hour postchallenge glucose level and upon repeat testing many with newly detected type 2 diabetes are found to "revert" to either impaired glucose tolerance or normal (37-39). We also had only single measures of both cystatin-C and glucose. This random error would serve however, to bias our results towards the null. The strengths of the study include the selection of a community-based population, detailed measures of several important

covariates, and the assessment of possible intermediate factors including hypertension, smoking, insulin resistance, inflammation, and endothelial dysfunction. Our results, however, need to be replicated, particularly among minority populations. Although observational studies cannot prove causality, the magnitude of the effect size and statistical control for many covariates make our findings compelling and important.

Cystatin-C has been shown recently to predict incident coronary heart disease events (40), suggesting that the renal/heart disease connection may share common mechanisms. Our findings provide an important new avenue for future research suggesting that mild renal impairment may occur early in the natural history of diabetes. The possibility that cystatin C may also predict type 2 diabetes remains to be tested, but if proved correct, raises intriguing possibilities for prevention and treatment as it might be fruitful to look for markers of renal impairment early in the course of hyperglycemia that could be modified by lifestyle and hygienic measures.

ACKNOWLEGEMENTS

The authors would like to acknowledge Ms Mya Swanson, MS for her assistance with database management and statistical analyses.

Table 1. Selected Baseline characteristics among prediabetic cases and matched controls

	Cases n=91		Controls n=273		P-value
	Mean	SD	Mean	SD	
Age (years)	57.9	11.0	54.2	10.8	0.005
BMI (kg/m$^{2)}$	28.12	5.2	26.91	4.6	0.040
Waist (cm)	91.9	13.46	87.2	12.5	0.003
Abdominal height (cm)	21.2	3.2	20.1	3.2	0.005
Lifetime total pack years cigarettes	14.4	22.6	9.1	15.81	0.037
Physical Activity (METS in past 7 days)	259.1	46.3	262.4	49.8	0.574
Glucose (mg/dl)	94.2	4.3	90.4	5.4	<0.001
HOMA	3.45	1.70	2.95	1.57	0.011
ACR (mg/g)	12.06	37.48	7.32	10.45	0.256
Cystatin C (mg/l)	1.05	0.23	.90	0.15	<0.001
eGFR[1] (**ml per minute per 1.73 m^2**)	83.0	17.7	83.6	17.0	0.631
Serum creatinine (g/l)	0.89	0.18	0.88	0.17	0.579
	%		%		
Male Gender (%)	42.9		42.9		------
Whites (%)	95.6		95.6		------
Education >12 years (%)	59.3		71.1		0.038
Family history of diabetes (%)	42.9		26.7		0.005
Hypertension (%)	38.6		20.8		0.001
Family history of HTN (%)	39.3		35.7		0.549
Smoking (%)					0.017
Never	48.4		51.8		
Ever	51.6		48.2		
Drinking (%)					0.028
Abstainer	7.7		9.7		
Non-current	30.8		20.4		
Non-daily	50.5		64.7		
Daily	11.0		5.2		

------ Matching Variable

[1] $eGFR = 1.86 \; x \; (serum\ creatinine)^{-1.154} \; x \; (Age)^{-0.203} \; x \; (0.742 \text{ if female}) \; x \; (1.21 \text{ if black})$

Table 2. Age-adjusted Odds ratios (95% CI) for Progression to Prediabetes According to Selected Baseline Characteristics

	Cases		Controls		OR†	95% CI†
	N	%	N	%		
BMI (kg/m^2)						
<27	43	47.8	161	60.3	1.00	
≥27	47	52.2	106	39.7	1.62	0.99, 2.66
Family history of diabetes:						
No	48	57.1	189	73.3	1.00	
Yes	36	42.9	69	26.7	1.85*	1.12, 3.07
Smoking:						
Never	34	37.4	141	51.8	1.00	
Ever	57	62.6	131	48.2	1.95*	1.15, 3.07
Current alcohol drinker:						
No	35	38.5	81	30.1	1.00	
Yes	56	61.5	188	69.9	0.65	0.38, 1.12
Hypertension:						
No	54	61.4	213	79.2	1.00	
Yes	34	38.6	56	20.8	2.39**	1.35, 4.22
Physical Activity (Total METS in past 7 days)						
< Median	52	57.1	136	50.0	1.00	
≥ Above median	39	42.9	136	50.0	0.82	0.49, 1.37
HOMA-IR						
1 (low)	15	17.0	90	33.2	1.00	
2	35	39.8	91	32.6	2.19*	1.12, 4.28
3 (high)	38	42.2	90	33.2	2.75*	1.37, 5.54
ACR (mg/g)						
<20	78	92.9	232	94.3	1.00	
≥20	6	7.1	14	5.7	0.94	0.34, 2.60
eGFR						
< median	52	57.1	134	49.1	1.00	
≥ median	39	42.9	139	50.9	0.84	0.50, 1.40
Serum Creatinine (g/l)						
< median	39	42.9	130	47.6	1.00	0.66, 2.11
≥ median	52	57.1	143	52.4	1.18	

†OR and 95% CI from Conditional Logistic Regression adjusted for age. Cases and controls matched on year of baseline interview, gender, race, and year of study enrollment.

* p<.05, ** p<.01

Table 3. Multivariate Odds ratios (95% CI) for Progression to Prediabetes According to Baseline Quintile of Cystatin-C (top 20% vs lowest 80%).

	Cases		Controls		Model 1		Model2	
	N	%	N	%	OR†	95% CI†	OR†	95% CI†
Cystatin-C (mg/l)								
Lowest 80%	42	46.7	218	79.9	1.00		1.00	
Top 20 %	48	53.3	55	20.1	5.08***	2.69, 9.58	3.04**	1.34, 6.89

†OR and CI from conditional logistic regression using matched cases and controls.
All models are conditioned on sex, race, and year of study enrollment.

Model 1 adjusted for age.
Model 2 adjusted for age, BMI (kg/m^2), family history of diabetes (no,yes), current smoker (never,ever), lifetime pack-years, fasting glucose (mg/dl), current drinker (no,yes), baseline hypertension(no,yes), HOMA-IR, and eGFR

** p<.01, *** p<.001

Table 4. Spearman Correlation Coefficients Between Cystatin-C and Selected Biomarkers for Inflammation and Endothelial Dysfunction Among the Control Group

	Cystatin-C
Age	0.377***
Hs-CRP	0.196**
E-selectin	0.179**
sICAM	0.294**
IL-6	0.243**
Serum creatinine	0.446***
eGFR	-0.384**
ACR	-0.007

** p< 0.01
*** p< 0.001

References

1. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346;393-403.

2. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Llanne-Parikka P, Keinanen-Kiukaanniem S, Laakso M, Louheranta A, Rastas M, Salminen V, Uustiupa M. Finnish Diabetes Prevention Study Group: Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-1350.

3. Editorial. Targeting people with pre-diabetes. BMJ 325; 403-404;2002.

4. Benjamin SM, Valdez R, Geiss LS, Rolka D, Venkat Narayan KM. Estimated number of adults with prediabetes in the US in 2000. Diabetes Care 2003; 26: 645-649.

5. Donahue RP, Orchard TJ. Diabetes mellitus and macrovascular complications: an epidemiologic perspective. Diabetes Care 1992;15:1141-1155.

6. Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as a predictor of vascular disease in non-diabetic subjects. Lancet 2;530-533: 1988.

7. Kuusisto J, Mykkanen L, Pyorala K, Lasskso M. Hyperinsulinemic microalbumiauria: a new risk indicator for coronary heart disease. Circulation 91;831-837: 1995.

8. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, et al. Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetelogia 32;219-226: 1989.

9. Klein R, Llein BEK, Linton KLP, Moss SE. Microalbuminuria in a population-based study of diabetes. Arch Intern Med 152;153-158:1992.

10. Mykkanen L, Zaccaro DJ, Wagenknecht LE, Robbins DC, Gabriel M, Haffner SM. Microalbuminuria is associated with insulin resistance in nondiabetic subjects. Diabetes 47; 793-800: 1998.

11. Stenhower CD, Henry RM, Dekker JM, Nippels G, Heine RJ, Buter LM. Microalbuminuria is associated with impaired brachial artery flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction. The Hoorn Study. Kidney Intl 2004; (Suppl) 92; S42-44.

12. Ochodnicky P, Vettoretti S, Henning RH, Bulkema H, Van Dokkum RP, de Zeew D. Endothelial dysfunction in chronic kidney disease: determinant of susceptibility to end-organ damage and therapeutic response. J Nephrol 19;246-258: 2006.

13. Ritz E. Minor renal dysfunction: an emerging independent cardiovascular risk factor. Heart 2003:89;963-964.

14. Knowler WC, Narayan KMV, Hanson RI., Nelson RG, Bennett PH. Tuomeilehto J, et al. Preventing non-insulin-dependent diabetes mellitus. Diabetes 1995;44:483-488.

15. Laterza OF, Price CP, Scott MG. Cystatin-C: an improved estimator of glomular filtration rate? Clin Chem 2002;48:699-707.

16. Tan GD, Lewis AV, James TH, Altmann P, Taylor RP, Levy JC. Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes. Reproducibility and accuracy compared with standard measures and iohexol clearance. Diabetes Care 25:2004-2009; 2002.

17. Wattanakit, K., Coresh, J., Muntner, P., Marsh, J., Folsom, A. R. Cardiovascular Risk Among Adults With Chronic Kidney Disease, With or Without Prior Myocardial Infarction. JACC 48: 1183-1189; 2006.

18. Shlipak, M. G., Katz, R., Sarnak, M. J., Fried, L. F., Newman, A. B., Stehman-Breen, C., Seliger, S. L., Kestenbaum, B., Psaty, B., Tracy, R. P., Siscovick, D. S. Cystatin C and Prognosis for Cardiovascular and Kidney Outcomes in Elderly Persons without Chronic Kidney Disease. Ann Intern Med 145: 237-246; 2006.

19. Hankey, G. J. Potential New Risk Factors for Ischemic Stroke: What Is Their Potential? Stroke 37: 2181-2188; 2006.

20. Bibbins-Domingo, K., Chertow, G. M., Fried, L. F., Odden, M. C., Newman, A. B., Kritchevsky, S. B., Harris, T. B., Satterfield, S., Cummings, S. R., Shlipak, M. G. Renal Function and Heart Failure Risk in Older Black and White Individuals: The Health, Aging, and Body Composition Study. Arch Intern Med 166: 1396-1402; 2006.

21. Amann, K., Wanner, C., Ritz, E. Cross-Talk between the Kidney and the Cardiovascular System. J Am Soc Nephrol 17: 2112-2119; 2006.

22. Stranges S, Wu T, Dorn JM, Freudenheim JL, Muti P, Farinaro E, Russell M, Nochajski TH, Trevisan M. Relationship of alcohol drinking pattern to the risk of hypertension: a population-based study. Hypertension;44:813-819; 2004.

23. Stranges S, Trevisan M, Dorn JM, Dmochowski J, Donahue RP. Body fat distribution, liver enzymes, and risk of hypertension. Evidence from the Western New York Study. Hypertension 2005; 46: **1-8.**

24. Chobanian AV, Bakrisk GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Jr, Roccella EJ. National Heart Lung and Blood Institute Joint National Committee on Prevention; National High blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 289:2560-2572; 2003.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: homeostasis model assessment: insulin resistance and β-cell function form fasting glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985.

26. Bakker AJ. Detection of microalbuminuria: receiver operating curve analysis favors albumin-to-creatinine ratio over albumin concentration. Diabetes Care 1999:22; 307-313

27. Levy AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol 2000;11: Suppl: 55A. abstract.

28. Levy AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461-470.

29. Koenig W, Twardella D, Brener, Rothenbacher D. Plasma concentrations of cystatin-C in patients with coronary heart disease and risk for secondary cardiovascular events: More than simply a marker of glomerular filtration rate. Clinic Chem 2005;51:321-327.

30. Microalbuminuria Collaborative Study Group, United Kingdom: Risk factors for development of microalbuminuria in insulin dependent diabetic patients: a cohort study. Br Med J 306:1235-1239;1993.

31. Haffner SM, Stern MP, Gruber MK, et al. Microalbuminuria. Potential marker for increased cardiovascular risk factors in nondiabetic subjects? Arteriosclerosis 10: 727-731; 1990.

32. Groop L, Ekstrand A, Forsblom C, et al. Insulin resistance, hypertension and microalbuminuria in patients with type 2 (non-insulin dependent diabetes mellitus. Diabetologia 36: 642-647; 1993.

33. Mykkanen L, Zaccaro DJ, Wahgenknecht LE, et al. Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the Insulin Resistance Atherosclerosis Study. Diabetes 1998;47:793-800.

34. Shlipak MG, Praught ML, Sarnak MJ. Update on cystatin C: new insights into the importance of mild kidney dysfunction. Curr Opin Nephrol Hypertens 2006; 15(3):270-275.

35. Shiplak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, et al. Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation 2003;107:87-92.

36. de Vegt F, Dekker JM, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. The 1997 American Diabetes Association criteria versus the 1985 World Health Organization criteria for the diagnosis of abnormal glucose tolerance: poor agreement in the Hoorn Study. Diabetes Care 1998; 21:1686-1690.

37. Feskens EJ, Bowles CH, Kromhout D. Intra- and interindividual variability of glucose tolerance in an elderly population. J Clin Epidemiol 1991;44:947-953.

38. McDonald DW, Fisher GF, Burnham C. Reproducibility of the oral glucose tolerance test. Diabetes 1965;14:473-480.

39. Olefsky JM, Reaven GM. Insulin and glucose responses to identical oral glucose tolerance tests performed forty-eight hours apart. Diabetes 1974;23:449-453.

40. Shiplak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, Siscovik DS, Stehman-Breen C. Cystatin C and the risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049-2060.